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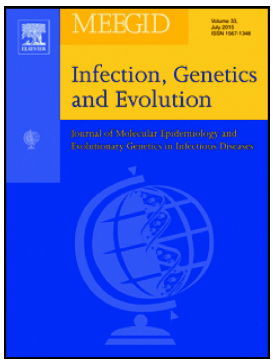

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Identification and characterization of a novel subtype of Tula virus in *Microtus arvalis obscurus* voles sampled from Xinjiang, China

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Abstract

Although most of *Arvicolinae* associated hantaviruses can not cause disease in humans, hemorrhagic fever with renal syndrome (HFRS) cases caused by Tula virus (TULV) have

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been described in Europe since 2002. In addition to Europe, TULV was also identified in the *Microtus arvalis obscurus* voles sampled from Kazakhstan, which shares borders with China. To gain more insight into the molecular epidemiology of TULV, a total of 365 rodents representing 7 species of 4 subfamily (*Arvicolinae*, *Murinae*, *Gerbillinae*, and *Cricetinae*) were captured in Qapqal county, Xinjiang, northwest China. Hantavirus RNA was recovered from 40 lung tissue samples of *M. arvalis obscurus*, with the prevalence of 10.96%. Genetic analysis revealed that all recovered viral sequences were most closely related to those of TULV, but exhibited > 11% nucleotide differences from all currently known TULV, suggesting that they may represent a new subtype of TULV. In the S tree, the newly identified viruses formed a distinct lineage and showed a close evolutionary relationship with those sampled from Southwestern Siberia and Kazakhstan. However, they exhibited a different clustering pattern in both the M and the L trees, suggesting the possibility of genetic reassortment. Finally, the recombination event was also observed in Xinjiang TULV viruses. In sum, all these data reveal a complex evolutionary history of TULV in Central Asia.

Keywords: Tula virus, *Microtus arvalis obscurus* vole, Recombination, Evolution, China.

1. Introduction

Orthohantaviruses (genus *Orthohantavirus*, family *Hantaviridae*) are characterized by a tripartite single-stranded RNA genome including small (S), medium (M), and large (L) segments, which encode a nucleocapsid protein (N), two external glycoproteins (Gn and Gc) and an RNA-dependent RNA polymerase, respectively (King et al., 2018). As an important zoonotic pathogen, rodent-borne orthohantaviruses cause two severe human diseases: hemorrhagic fever with renal syndrome (HFRS) in the Old World, and hantavirus pulmonary

syndrome (HPS) in the New World, with case fatality rates up to 15% and 40%, respectively (Holmes and Zhang, 2015; Schmidt-Chanasit et al., 2010; Watson et al., 2014). In the Old World, Dobrava-Belgrade virus (DOBV), Hantaan virus (HTNV), Seoul virus (SEOV) and Puumala virus (PUUV) are the major pathogens of HFRS (Nikolic et al., 2014; Plyusnin et al., 1995; Zhang et al, 2010). These viruses are primarily hosted by yellow-necked mouse (*Apodemus flavicollis*), striped field mouse (*Apodemus agrarius*), Norway rats (*Rattus norvegicus*), and bank vole (*Myodes glareolus*), respectively. However, to date, for the majority of *Arvicolinae* associated hantaviruses [e. g. Prospect Hill virus (PHV), Bloodland Lake virus (BLLV), Isla Vista virus (ISLAV) and Khabarovsk virus (KHAV)], no serious illness or death in humans caused by them has been described (Jiang et al., 2017; Schmidt-Chanasit et al., 2010). Tula virus (TULV) was first identified in European common voles (*M. arvalis and M. rossiaemeridionalis*) sampled from Tula region of Central Russia in 1994 (Plyusnin et al., 1994). Currently, TULV has been found in both Europe and Central Asia (Polat et al., 2019). In addition to *M. arvalis* and *M. rossiaemeridionalis*, TULV has also been found in multiple species of rodents, e. g. *M. subterraneus*, *M. agrestis*, *M. gregalis*, *Arvicola amphibius* and *Lagurus lagurus* (Schmidt-Chanasit et al., 2010). In sum, all these data indicate a wide geographic distribution and a broad range of animal hosts of TULV. Importantly, HFRS cases caused by TULV have been described in Europe since 2002 (Clement et al., 2003; Klempa et al., 2003; Reynes et al., 2015; Schultze et al., 2002; Zelená et al., 2013), revealing the implication of TULV for the public health.

Xinjiang, which is a provincial-level autonomous region of China, is located in the northwest of the country (Fig. 1). Although the numbers of HFRS cases and death in China

remain the highest in the world, no HFRS cases have been described in Xinjiang so far (Zhang et al, 2010; Jiang et al., 2017). To date, SEOV harbored by the brown rat has been described in Xinjiang province, and is considered to have been originated from other Chinese endemic regions of hantaviruses following the migration of its host (Guo et al., 2016). Recently, one study described the detection of TULV in *Microtus arvalis obscurus* voles sampled from Xinjiang (Guo et al., 2019). However, rodents in Xinjiang exhibit a high diversity, with more than 68 species (Wang et al., 1983). Among those rodents, 19 species belong to the subfamily *Arvicolinae* (Wang et al., 1983), suggesting the possible presence of other hantaviruses in native rodents in Xinjiang. Herein, we performed a molecular epidemiologic investigation in Qapqal county, which is located in the western part of Xinjiang (Fig. 1) and borders Kazakhstan. Consequently, a new subtype of TULV was identified in *M. arvalis obscurus* voles sampled from Xinjiang and its genetic features were characterized in this study.

2. Materials and methods

2.1. Collection of animals

During 2016, 365 rodents were trapped in Qapqal county of Yili prefecture of Xinjiang Uygur Autonomous Region, China (Fig. 1). All captured rodents were firstly identified by experienced biologists, and then determined by analyzing sequence of mitochondrial cytochrome *b* (mt-*cyt* b) gene (Guo et al, 2013). To minimize suffering, animals were anesthetized with ether before surgery as previously described (Guo et al, 2013), and then lung tissue samples were collected and stored at -80 °C.

2.2. Extraction of DNA and RNA, RT-PCR and sequencing

According to the manufacturer's instruction, total DNA and RNA were extracted from each of 365 lung tissue samples using DNA or RNA isolation kit respectively (Omega biotek, USA). Total RNA was reverse transcribed using one step RT-PCR kit (TaKaRa, Dalian, China). Hantaviral RNA was detected by nested RT-PCR as described previously (Klempa et al., 2006; Wang et al., 2014). To recover the complete genomes, primers were designed based on several known and related genome sequences of hantaviruses (Guo et al, 2013), and were described in Table S1. The entire coding sequences of S, M, and L segments were obtained by nested RT-PCR, while the 5'and 3' ends were obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) using a RACE kit (TaKaRa, Dalian, China). Simple PCR was used to amplify mt-*cyt* b gene as described previously (Guo et al, 2013).

QIAquick Gel Extraction kit (Qiagen, Valencia, USA) was used to purify the PCR products before sequencing. Purified DNA with < 700bp was sequenced directly, while those with >700 bp was firstly cloned into pMD18-T vector (TaKaRa, Dalian, China), and then transformed into JM109-143 competent cells. For each sample, at least three clones were selected for sequencing.

Complete viral genome (strains Xinjiang-7, -10, -33, and -198) and mt-*cyt* b gene sequences have been deposited in GenBank under accession numbers MN052670-MN052673 for the S segment, MN183137- MN183140 for the M segment, MN183133-MN183136 for the L segment, and MN183141- MN183144 for mt-*cyt* b, respectively.

2.3. Recombination analysis

To find possible recombination events in S segment of TULV sampled from Xinjiang, the

RDP, GENECONV, bootscan, maximum chi square, Chimera, SISCAN, and Distance Plot recombination detection methods in RDP4 (Martin et al., 2015) were used to detect potential recombinant viral sequences, identify likely parental viral sequences, and localize possible recombination breakpoints (Lin et al, 2012). The analyses were performed with default settings for the different test methods and a Bonferroni corrected P value cutoff of 0.05. In addition, Simplot version 3.5.1 (Lole et al., 1999) was also used to analyze sequence alignment with similarity plot and bootscan analysis methods, with a window size of 250 nt and a step size of 20 nt. Finally, when events were found with two or more methods and with significant phylogenetic support, the hantaviral sequences were considered recombinant in this study.

2.4. Phylogenetic analysis

Hantaviruses sequences (53 S, 26 M and 20 L segment sequences) and mt-*cyt* b gene (52 sequences) obtained in this study, as well as those retrieved from GenBank (Table S2-S3), were aligned using the Clustal W method implemented in the MEGA program, version 6.0 (Tamura et al., 2013). Sequences identities of nucleotide (nt) and amino acid (aa) were calculated by MegAlign program available within the DNASTAR Lasergene package, version 5 (DNASTAR, Inc., Madison, WI).

Phylogenetic trees were reconstructed by using the maximum likelihood (ML) method available within the IQ-TREE program under the best-fit substitution model (Kalyaanamoorthy et al., 2017; Trifinopoulos et al., 2016), with ultrafast bootstrap support values calculated from 1,000 replicate trees. Bootstrap values higher than 70% were considered significant. In addition, the MCMC method was used to estimate the Bayesian

trees of mt-*cyt* b gene via MrBayes software (Huelsenbeck and Ronquist, 2001), and the best-fit model was determined by jModelTest (Posada, 2008).

3. Results

3.1. Detection of hantavirus in rodents.

During 2016, a total of 365 rodents were captured in Qapqal county of Yili prefecture of Xinjiang Uygur Autonomous Region, northwest China (Fig. 1). These rodents represent 7 species of 4 subfamily (*Arvicolinae*: 259 *M. arvalis obscurus*, 2 *Ellobius talpinus*; *Cricetinae*: 5 *Cricetulus migratorius*; *Gerbillinae*: 14 *Rhombo mysopimus*, 6 *Meriones tamariscinus*, 5 *Meriones libycus*; and *Murinae*: 74 *A. sylvaticus*) (Table S4). Nested-RT-PCR targeting the partial L segment sequence (Guo et al., 2011; Klempa et al., 2006) was performed to screen hantaviral RNA in 365 lung tissue samples. Consequently, PCR products of the expected size (396 nt) were recovered from 40 *M. arvalis obscurus*. Genetic analysis revealed that all recovered sequences are most closely related to TULV (see below), with an overall positive rate of 15.44 % in *M. arvalis obscurus* voles, indicating the circulation of TULV in Xinjiang.

3.2. Genetic analysis of the newly-identified TULV

To characterize the hantaviruses harbored by *M. arvalis obscurus* voles sampled from Xinjiang, the complete S, M and L segments were successfully recovered from 4 positive vole samples (designated strains as 'Xinjiang-7, -10, -33, -198'). Genetic analysis of the complete S, M and L segments of these four newly identified viruses revealed that they were closely related each other respectively, with less than 3% nt divergence. Comparison with all known hantaviruses revealed that all newly identified viruses belong to TULV. Their genetic features of the S, M and L segments were described in Table S5. The complete S segment sequences

comprises of 1,830 nt, including 43 nt in the 5'NCR, 1,293 nt of ORF encoding the N protein of 430 aa residues, and 494-nt-long 3'NCR. Notably, the coding region of the S segment sequences were particularly close to the strain xj4, which was also identified in *M. arvalis obscurus* voles sampled from Xinyuan county of Yili prefecture (Guo et al., 2019), close to Qapqal county, with 88.5-88.9% nt and 98.0-98.3% aa identities. However, all Xinjiang viruses identified here and previously exhibited more differences (82.9-92.2% nt and 95.2-98.2% aa identities) from those discovered in Europe and Kazakhstan (Table S6). A putative 90 aa long nonstructural protein (NSs) was found at the nt position 83-355 as the second ORF in the S segment sequences of Xinjiang viruses. Specific aa motif V253 was observed in Xinjiang-7, -10, -33, and -198 strains and aa motif T383 in Xinjiang-33, -198 strains, respectively. However, pairwise comparison with all known M and L segment sequences revealed that the newly identified TULV strains were far distant from those sampled from Central Europe and Turkey, with 80.1-81.9% and 78.6-81.1% nt identities and 95.7-97.8% and 95.4-96.4% aa identities for the coding region of the M and the L segment sequences, respectively (Table S7 and Table S8). In sum, these data suggested that the viruses identified in the *M. arvalis obscurus* voles from Xinjiang may represent a new subtype of TULV.

3.3. Phylogenetic analysis of novel TULV subtype

To gain more insights into TULVs in China, phylogenetic trees were reconstructed using ML methods based on the entire coding region of the S, M, and L segment sequences (Fig. 2). In all three phylogenetic trees, Xinjiang TULV sequences recovered in this study formed a distinct lineage, supporting newly identified strains as a new subtype. In the S tree (Fig. 2A),

all known TULVs were divided into ten lineages according to their geographic origins. Viral sequences within Chinese lineage showed the closest evolutionary relationship with those sampled from Southwestern Siberia (AF442621) and then Kazakhstan (AM945877) respectively. In addition, they were more closely related to those identified in *M. arvalis* and *M. rossiaemeridionalis* voles sampled from Central Russia (Z30942- Z30945). Remarkably, they showed a close evolutionary relationship to those sampled from central Europe in the M segment tree (Fig. 2B), while they showed a close relationship with the viral sequences sampled from Turkey (MH649271) in the L segment tree (Fig. 2C), suggesting the possible occurrence of genetic reassortment of these Xinjiang viruses.

3.4. Recombination analysis of TULVs discovered in Xinjiang

Both RDP4 and Simplot methods were used to perform recombination analysis of TULVs sampled from Xinjiang. No recombination events were found in the newly identified strains (strains Xinjiang-7, -10, -33, and -198). However, a significant recombination event was observed in the S segment sequence of the strain xj4 identified in Xinjiang previously (Guo et al., 2019) (Fig. 3). Two recombination breakpoints were observed at positions nt 552 and 924 of the S segment sequence (with reference to the Xinjiang-33 sequence), so that the S segment sequence were divided into three regions (Fig. 4), grouped as two putative "parental regions". In parental region A (nt 552 to 924), the strain xj4 had 90.0% nt similarity to those of the newly-identified Chinese strains, as opposed to 80.0% nt similarity to Kazakhstan strains. However, in other two regions (nt 1 to 551 and nt 925 to 1233), the strain xj4 has higher similarity (97.6% nt) to Kazakhstan strains than to Chinese strains (87.6% nt). Phylogenetic analyses also provided a strong evidence for the recombination event (Fig. 4), suggesting the

occurrence of the recombinant event for the strain xj4.

3.5. Phylogenetic analysis of rodent hosts sequences

M. arvalis voles are widespread across Eurasia and include several subspecies. *M. arvalis obscurus* vole is considered as a subspecies of *M. arvali* (Wilson and Reeder, 2005). To better understand the association between the newly identified variant of TULV and their hosts, complete coding sequences of mt-*cyt* b gene were amplified from four Xinjiang voles from which complete viral genome sequences were recovered. Phylogenetic analysis of the mt-*cyt* b gene sequences revealed that *M. arvalis obscurus* voles are clearly different from *M. arvalis* voles circulating in Europe (Fig. 5). In addition, *M. arvalis obscurus* voles are divided into two groups. One is comprised of the voles from China and Kazakhstan, while another is comprised of those from Turkey. Notably, the voles from Kazakhstan were closely related to those from Xinjiang of China (Fig. 5).

4. Discussion

Although rodents exhibit a high diversity in Xinjiang, hantaviruses were identified just in the brown rats sampled from Tulufan city in 1980s and Ürümqi city (the capital of Xinjiang) in 2016 (Guo et al, 2016). Notably, both cities are linked each other and to Chinese inland regions by Lanzhou-Xinjiang Railway line. It is believed that the brown rats in Xinjiang were migrated from the Chinese inland regions following the Lanzhou-Xinjiang Railway line (Guo et al, 2016). Therefore, SEOV identified in Xinjiang (Guo et al, 2016) might have been originated from the neighboring epidemic areas of HFRS. Notably, TULV also was found in Xinjiang recently (Guo et al, 2019). This study demonstrates the circulation of a novel variant of TULV in *M. arvalis obscurus* voles in Yili prefecture of Xinjiang Uygur Autonomous

Region of China. In addition to the hosts of TULV, there are other 68 rodent species including 7 species of the subfamily *Murinae* and 19 species of the subfamily *Arvicolinae* in Xinjiang (Wang et al., 1983). Of these rodents, *A. agrarius*, *M. rufoucanus* and *M. rutilus*, are known to harbor Hantaan virus and Hokkaido virus, respectively, tentatively suggesting that both viruses may also be present in Xinjiang. In sum, all these data are suggestive of considerable genetic diversity of hantaviruses in Xinjiang. Due to the important geography of Xinjiang in Eurasia, more efforts are needed to investigate the diversity of hantaviruses of Xinjiang.

TULV was initially identified in European common voles *M. arvalis* and *M. rossiaemeridionalis* sampled from Tula region of Russia (Plyusnin et al., 1994). Since then, the virus has been identified in a broad range of *Microtus* voles, and even in other genera (*A. amphibious* and *L. lagurus*) (Plyusnina et al., 2008; Song et al., 2002; Schmidt-Chanasit et al., 2010; Schlegel et al., 2012). Thus, it is considered that TULV is less host-specific (Schmidt-Chanasit et al., 2010; Schlegel et al., 2012). Such multi-host association is also observed in SEOV and their rat hosts (Zhang et al, 2010; Lin et al, 2012). However, like other hantaviruses, TULVs cluster according to their geographic origins (Plyusina et al., 2008; Schmidt-Chanasit et al., 2010). In this study, the viruses identified in *M. arvalis obscurus* formed a distinct lineage in the S tree (Fig. 2A), and showed a closer evolutionary relationship with those identified in *M. gregalis* sampled from Omsk of Russia (AF442621) rather than those identified also in *M. arvalis obscurus* sampled from Karatal of Kazakhstan (Plyusina et al., 2008), even though Qapqal county is closer to Karatal of Kazakhstan than Omsk of Russia (Fig. 1). More importantly, the Xinjiang viruses were more closely related to those sampled from Central Europe in the M tree (Fig. 2B), but to those sampled from Turkey

in the L tree (Fig. 2C). All these data are suggestive of a complex evolutionary history of TULV in Xinjiang and neighboring regions.

Both recombination and reassortment play a significant role in shaping patterns of genetic diversity in RNA viruses (Holmes, 2009). They have been found in different families of RNA viruses (Shi et al, 2016). The first evidence of homologous recombination within the S segment of hantavirus was identified in TULV viruses sampled from East Slovakia, where mosaic-like structure of the S segment was observed, corresponding to several recombination events (Sibold et al., 1999). Evidence for TULV recombination was also observed in two Serbian lineages (Nikolic et al., 2014). Moreover, the recombinant TULVs within the S segment was also generated in experimental conditions (Plyusnin et al.,2002). As shown in Fig. 3 and Fig. 4, the strain xj4 (KX270414), which was identified in Narat grassland of Xinyuan county of Yili prefecture seems to be a recombinant between the TULV variant described here and those found in Kazakhstan (Plyusnina et al., 2008). Although Qapqal county and Xinyuan county are close to each other, and are far from Karatal of Kazakhstan, two regions of the S segment of the strain xj4 are closely related to the Kazakhstan strain rather than the newly identified viruses sampled from Qapqal county. These data strongly support the occurrence of genetic recombination the virus. Finally, the different clustering patterns of the newly identified TULVs in Xinjiang also suggest the genetic reassortment. In sum, all these data support a complex evolutionary history of TULV in the Central Asia. Hence, more efforts will be helpful to better understand the diversity and evolution of TULV.

Conflict of interest statement

The authors declare no conflict of interest.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table S1. Primers used in this study.

Table S2. Reference sequences used for phylogenetic analyses in this study.

Table S3. Cytochrome *b* gene sequences of *Microtus arvalis obscurus*, *M. arvalis* and *Myodes glareolus*.

Table S4. Prevalence of hantavirus in rodents by species in Xinjiang, China.

Table S5. Genome organization of Tula virus (TULV) strains identified in this study and the corresponding prototype strains.

Table S6. Percentage similarities of S segments among the newly identified hantaviruses and known hantaviruses.

Table S7. Percentage similarities of M segments among the newly identified hantaviruses and known hantaviruses.

Table S8. Percentage similarities of L segments among the newly identified hantaviruses and known hantaviruses.

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Fig. 1. A map of Eurasia including China, Kazakhstan and Russia. The geographic locations in which rodents were captured and TULV was identified in this study (●) and (●) previously, and SEOV (●) identified previously in Xinjiang.

Fig. 2. ML phylogenetic trees of TULV based on the entire coding sequences of the S (A), M

(B) and L (C) segment. Only bootstrap values greater than 70% are shown.

Fig. 3. (a) Sequence similarity plot revealing two recombination breakpoints with the locations (shown by the red numbers on the x-axis). The plot shows genome scale similarity comparisons of the sequence of the strain xj4 (query) against the strains Taldykorgan343, Karatal340 and Karatal322 (parental group 1; blue) and the stains Xinjiang-7, -10, -33 and -198 (parental group 2; red). (b) Bootscan analysis of 1233 nt long of the S segment of TULV, as analyzed in Simplot (Window: 250 bp, Step: 20 bp, GapStrip: On, Reps: 100, Kimura (2-parameter), T/t: 2.0, Neighbor-Joining); The peak values exceeding 70% were considered to be significant.

Fig. 4. Phylogenies of parental region A (nt 552 to 924) and region B (nt 1 to 551 and 925 to 1233). Phylogenetic trees were reconstructed by using the maximum likelihood (ML) method available within the IQ-TREE program under the best-fit substitution model. The numbers (>70) above branches indicate percent bootstrap values.

Fig. 5. Bayesian phylogenetic tree of rodents based on mt-*cyt* b gene sequences. Posterior node probabilities greater than 0.7 are shown.

Highlights

- A new subtype of Tula virus was identified in *Microtus arvalis obscurus* from China.
- Recombination event was observed in the S segment of TULV from China.
- Phylogenetic analysis reveals a complex evolutionary history of TULV in Central Asia.

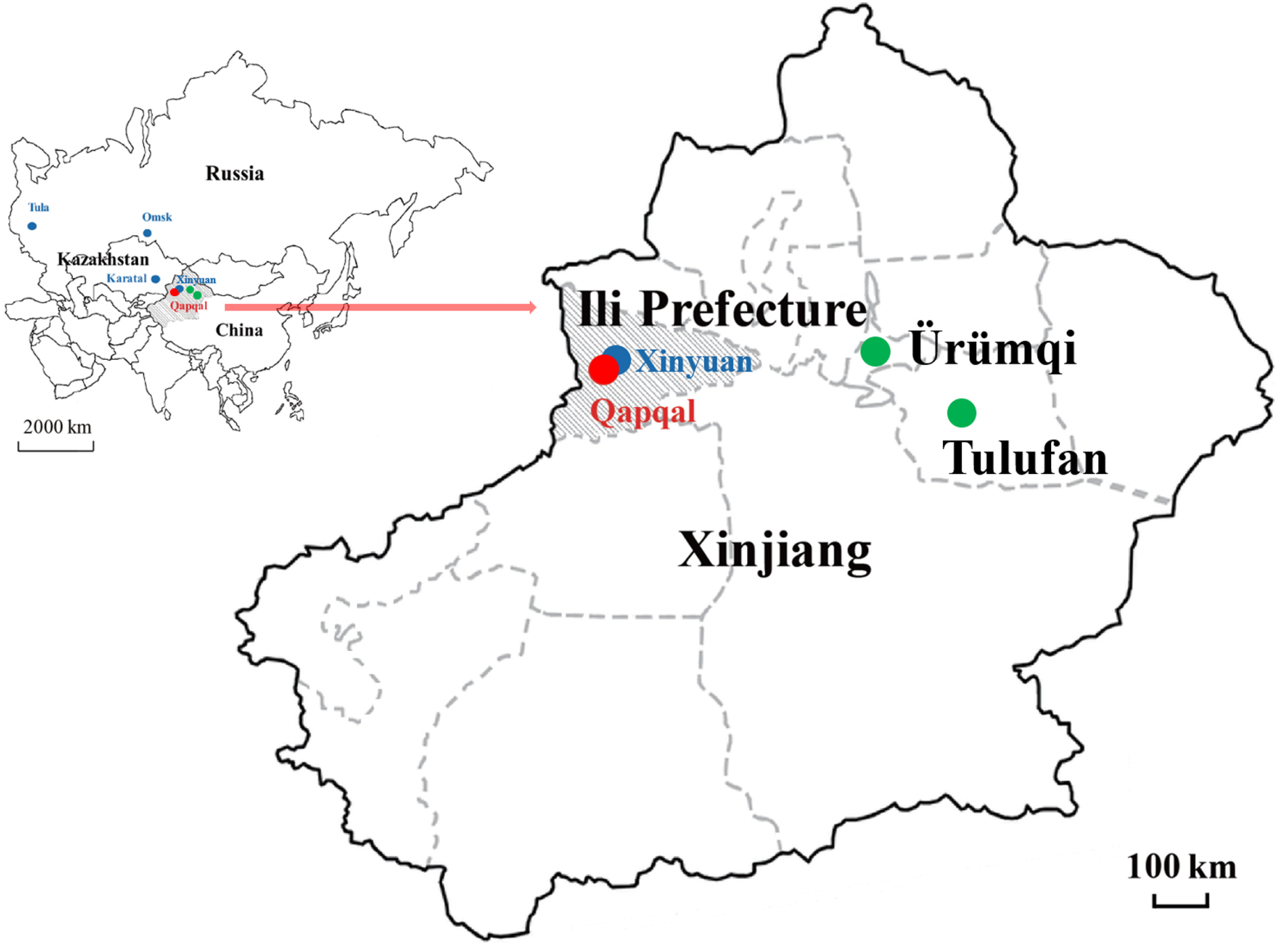
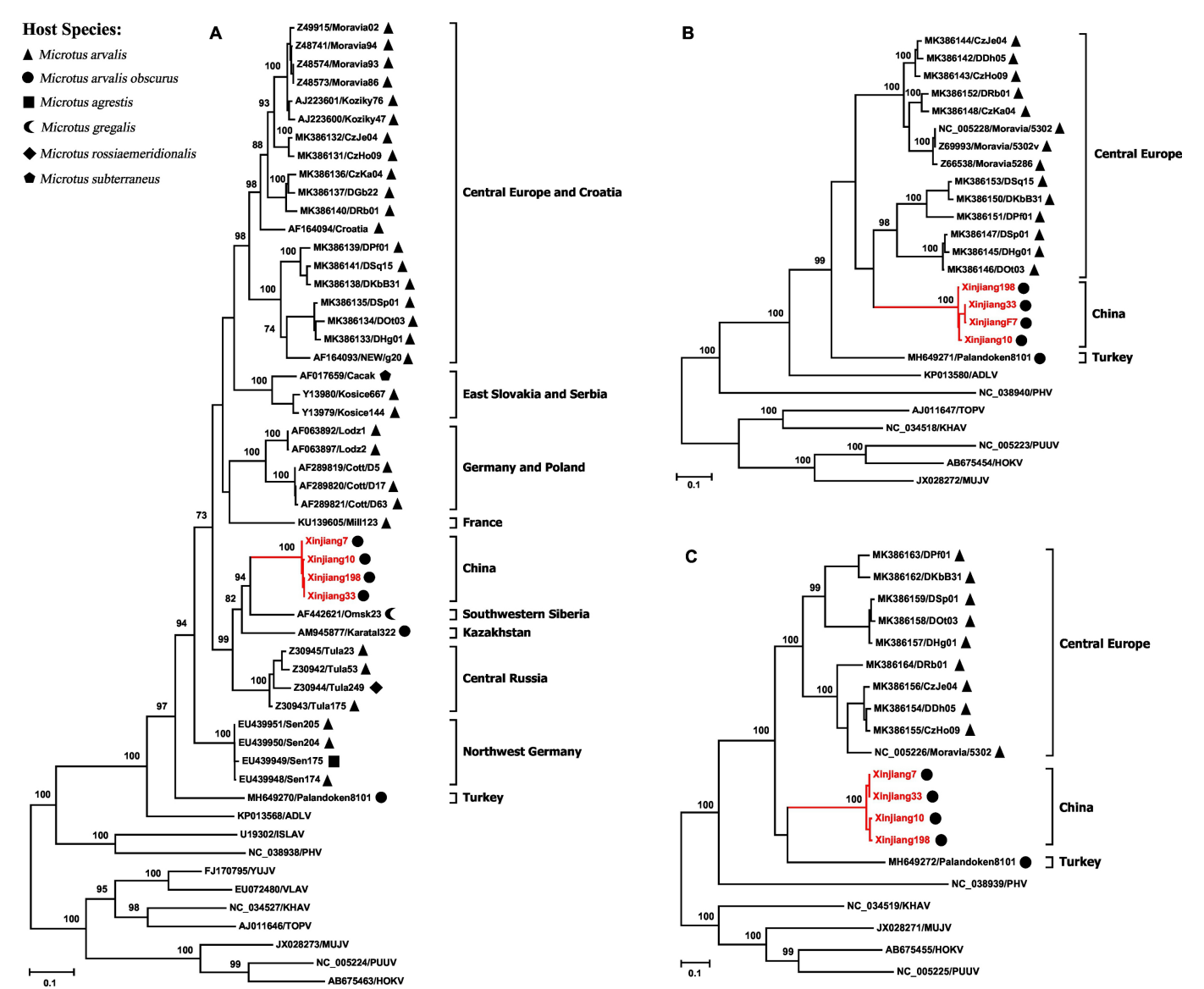

Russia
Tula
Omsk
Kazakhstan
Karatal
Xinyuan
Qapqal
China
2000 km
Ili Prefecture
Xinyuan
Qapqal
Ürümqi
Tulufan
Xinjiang
100 km

Figure 1


Host Species:
Microtus arvalis
Microtus arvalis obscurus
Microtus agrestis
Microtus gregalis
Microtus rossiaemeridionalis
Microtus subterraneus
A
Z49915/Moravia02
Z48741/Moravia94
Z48574/Moravia93
Z48573/Moravia86
AJ223601/Koziky76
AJ223600/Koziky47
MK386132/CzJe04
MK386131/CzHo09
MK386136/CzKa04
MK386137/DGb22
MK386140/DRb01
AF164094/Croatia
MK386139/DPf01
MK386141/DSq15
MK386138/DKbB31
MK386135/DSp01
MK386134/DOt03
MK386133/DHg01
AF164093/NEW/g20
Central Europe and Croatia
AF017659/Cacak
Y13980/Kosice667
Y13979/Kosice144
East Slovakia and Serbia
AF063892/Lodz1
AF063897/Lodz2
AF289819/Cott/D5
AF289820/Cott/D17
AF289821/Cott/D63
Germany and Poland
KU139605/Mill123
France
Xinjiang7
Xinjiang10
Xinjiang198
Xinjiang33
China
AF442621/Omsk23
Southwestern Siberia
AM945877/Karatal322
Kazakhstan
Z30945/Tula23
Z30942/Tula53
Z30944/Tula249
Z30943/Tula175
Central Russia
EU439951/Sen205
EU439950/Sen204
EU439949/Sen175
EU439948/Sen174
Northwest Germany
MH649270/Palandoken8101
Turkey
KP013568/ADLV
U19302/ISLAV
NC_038938/PHV
FJ170795/YUJV
EU072480/VLAV
NC_034527/KHAV
AJ011646/TOPV
JX028273/MUJV
NC_005224/PUUV
AB675463/HOKV
0.1
B
MK386144/CzJe04
MK386142/DDh05
MK386143/CzHo09
MK386152/DRb01
MK386148/CzKa04
NC_005228/Moravia/5302
Z69993/Moravia/5302v
Z66538/Moravia5286
MK386153/DSq15
MK386150/DKbB31
MK386151/DPf01
MK386147/DSp01
MK386145/DHg01
MK386146/DOt03
Central Europe
Xinjiang198
Xinjiang33
XinjiangF7
Xinjiang10
China
MH649271/Palandoken8101
Turkey
KP013580/ADLV
NC_038940/PHV
AJ011647/TOPV
NC_034518/KHAV
NC_005223/PUUV
AB675454/HOKV
JX028272/MUJV
0.1
C
MK386163/DPf01
MK386162/DKbB31
MK386159/DSp01
MK386158/DOt03
MK386157/DHg01
MK386164/DRb01
MK386156/CzJe04
MK386154/DDh05
MK386155/CzHo09
NC_005226/Moravia/5302
Central Europe
Xinjiang7
Xinjiang33
Xinjiang10
Xinjiang198
China
MH649272/Palandoken8101
Turkey
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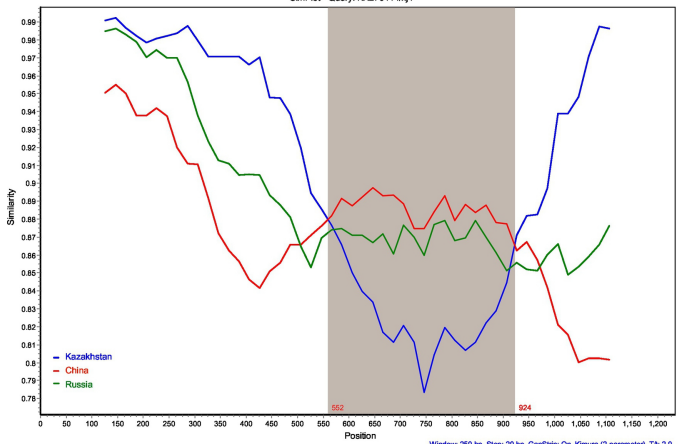
JX028271/MUJV
AB675455/HOKV
NC_005225/PUUV
0.1

Figure 2


SimPlot - Query: KX270414/xj4
Similarity
Position
Kazakhstan
China
Russia
552
924
Window: 250 bp, Step: 20 bp, GapStrip: On, Kimura (2-parameter), T/t: 2.0

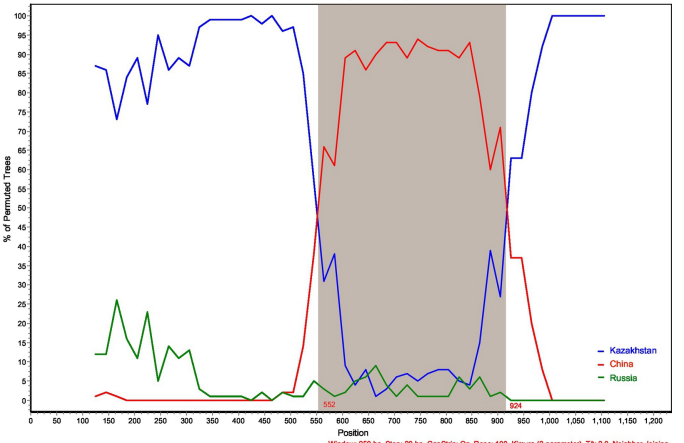
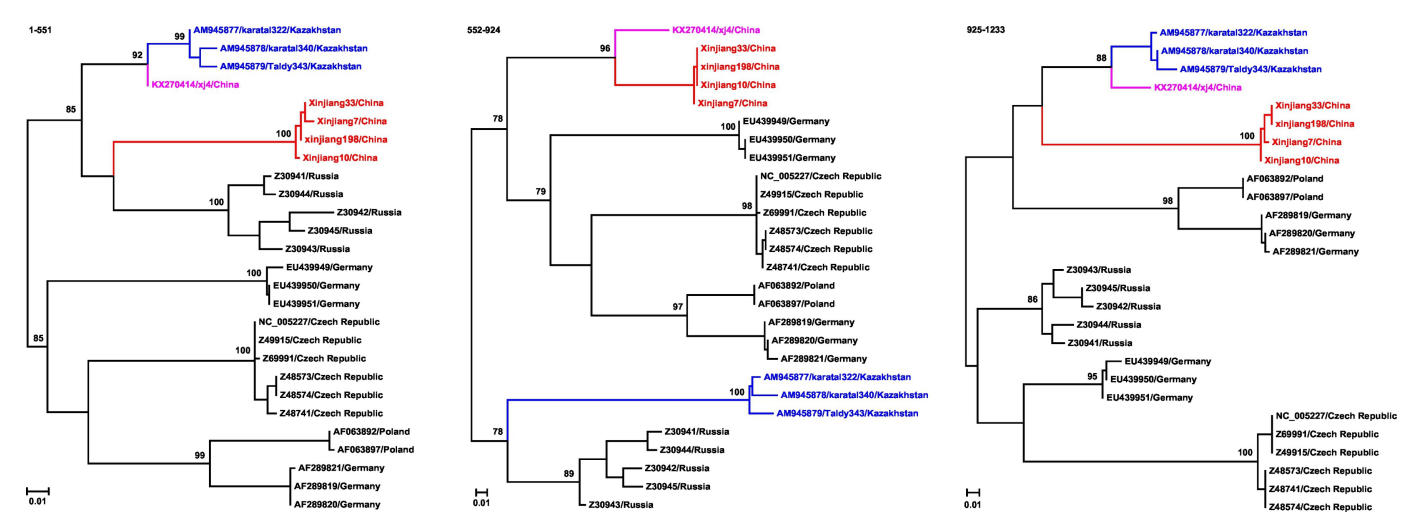

BootScan - Query: KX270414/xj4
% of Permuted Trees
Position
Kazakhstan
China
Russia
552
924
Window: 250 bp, Step: 20 bp, GapStrip: On, Reps: 100, Kimura (2-parameter), T/t: 2.0, Neighbor-Joining

Figure 3


1-551
AM945877/karatal322/Kazakhstan
AM945878/karatal340/Kazakhstan
AM945879/Taldy343/Kazakhstan
KX270414/xj4/China
Xinjiang33/China
Xinjiang7/China
xinjiang198/China
Xinjiang10/China
Z30941/Russia
Z30944/Russia
Z30942/Russia
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EU439949/Germany
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Z48574/Czech Republic
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AF063897/Poland
AF289821/Germany
AF289819/Germany
AF289820/Germany
0.01
552-924
KX270414/xj4/China
Xinjiang33/China
xinjiang198/China
Xinjiang10/China
Xinjiang7/China
EU439949/Germany
EU439950/Germany
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Z30941/Russia
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Z30943/Russia
0.01
925-1233
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KX270414/xj4/China
Xinjiang33/China
xinjiang198/China
Xinjiang7/China
Xinjiang10/China
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AF289821/Germany
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EU439950/Germany
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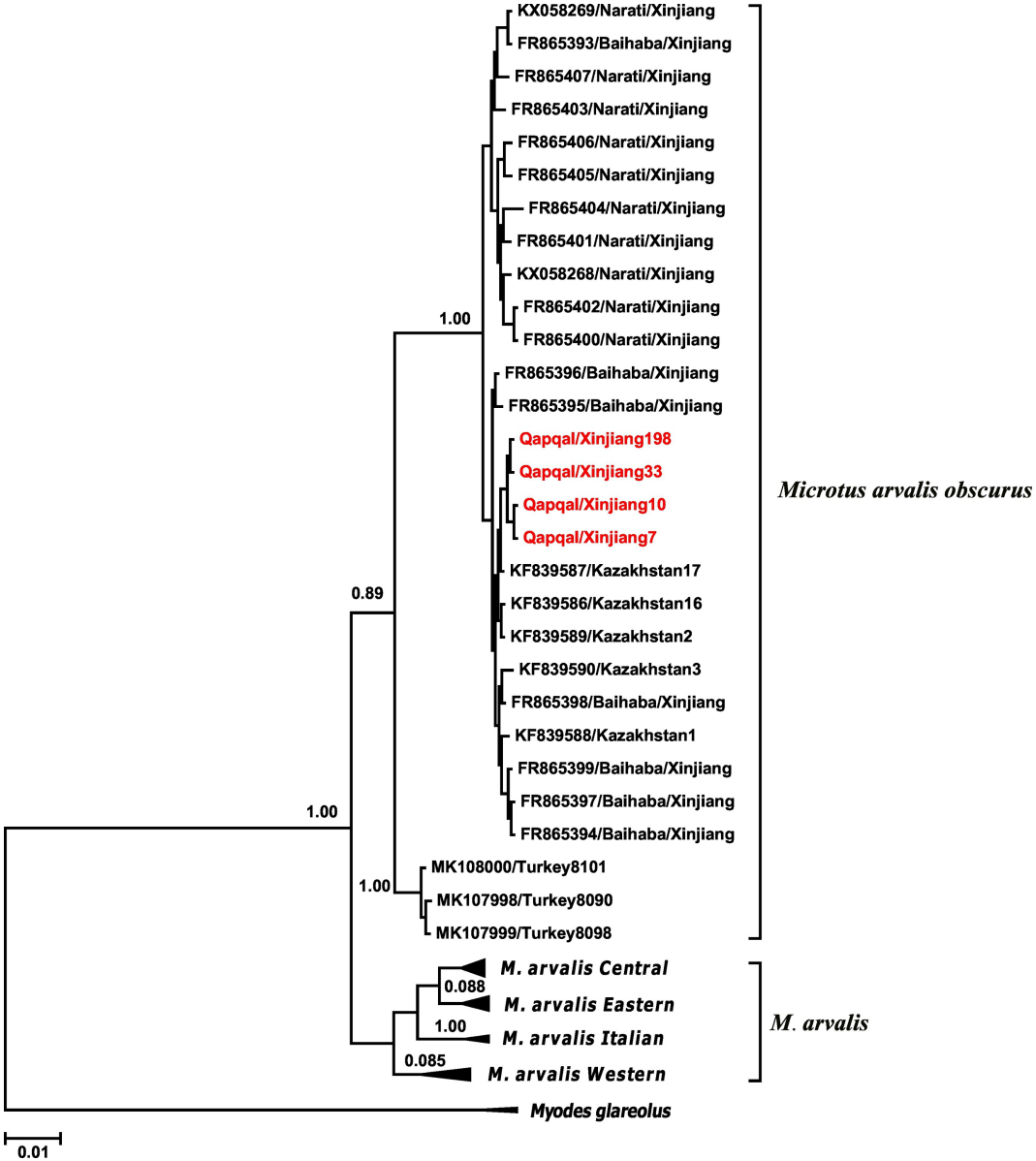
0.01

Figure 4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FR865403/Narati/Xinjiang
FR865406/Narati/Xinjiang
FR865405/Narati/Xinjiang
FR865404/Narati/Xinjiang
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FR865400/Narati/Xinjiang
FR865396/Baihaba/Xinjiang
FR865395/Baihaba/Xinjiang
Qapqal/Xinjiang198
Qapqal/Xinjiang33
Qapqal/Xinjiang10
Qapqal/Xinjiang7
KF839587/Kazakhstan17
KF839586/Kazakhstan16
KF839589/Kazakhstan2
KF839590/Kazakhstan3
FR865398/Baihaba/Xinjiang
KF839588/Kazakhstan1
FR865399/Baihaba/Xinjiang
FR865397/Baihaba/Xinjiang
FR865394/Baihaba/Xinjiang
MK108000/Turkey8101
MK107998/Turkey8090
MK107999/Turkey8098
M. arvalis Central
M. arvalis Eastern
M. arvalis Italian
M. arvalis Western
Myodes glareolus
1.00
0.89
1.00
1.00
0.088
1.00
0.085
Microtus arvalis obscurus
M. arvalis
0.01

Figure 5